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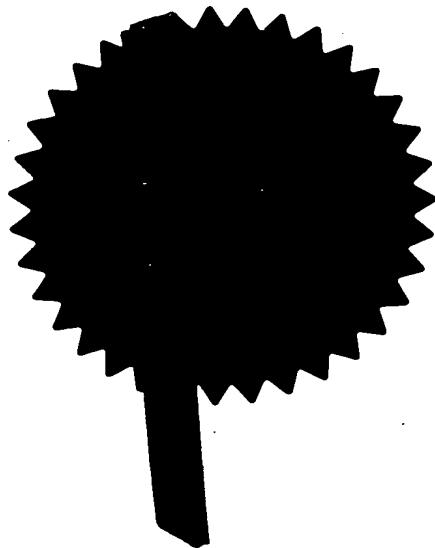
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**Stirling**  
**FK7 9JQ**

Patents ADP number (if you know it)

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## United Kingdom

4. Title of the invention	Diagnostic Test
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5. Name of your agent (if you have one) **Carpmaels & Ransford**

**"Address for service" in the United Kingdom  
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*Carpmaels & Ransford*

9th March 1999

Carpmaels & Ransford

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Paul N. Howard

0171 242 8692

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## DIAGNOSTIC TEST

The present invention is concerned with identification of proteins of the cytosolic phospholipase A<sub>2</sub> enzyme and the applications thereof, particularly in diagnosis, treatment  
5 monitoring and drug development.

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymes are generally characterised by their capacity to catalyse the hydrolysis of the Sn2 acyl-ester bond of glycerophosphate to release free fatty acid (Mayer R.J. and Marshall L., *FASEB J*, 7, 339-348; Dennis E.A., Ed., *Phospholipase*  
10 *A<sub>2</sub> Methods in Enzymology*, 1991, 197, 359-433). The scientific literature recognises different types of PLA<sub>2</sub> enzyme. Types I, II and III are referred to as secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) enzymes. Secretory phospholipase A<sub>2</sub> enzymes have a molecular weight of approximately 14 kDa, are found extracellularly and have been recognised in plasma.

15 The type IV PLA<sub>2</sub> enzyme is referred to as cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) and is characterised as a cellular messenger enzyme. The cPLA<sub>2</sub> enzyme occupies a key position in cellular signalling mechanisms as its activity regulates the supply and release of highly unsaturated fatty acids such as arachidonic acid from membrane phospholipids for  
20 subsequent action in cell messenger processes.

The cPLA<sub>2</sub> enzyme has been identified in a number of tissues including human monocytes (Clark J.D. *et al.*, *Cell*, 65, 1991, 1043-1051; Kramer R.M. *et al.*, *J. Biol. Chem.*, 266, 1991, 5268-5272) and human platelets (Takayama K. *et al.*, *FEBS*, 282, 1991, 326-  
25 330).

The amino acid sequence of cPLA<sub>2</sub> has been determined for the enzyme purified from human monocyte (U937) cells (Clark J.D. *et al.*, *Cell*, 65, 1991, 1043-1051). cPLA<sub>2</sub> of the type found in U937 cells has an amino acid sequence which is 749 amino acids long.  
30 This sequence shows a region of homology with a limited number of other proteins including protein kinase C (PKC), GTPase activating protein (GAP), phospholipase C and synaptic vesicle protein p65. The points of homology occur towards the N-terminal end of the protein in the so-called calcium-binding portion which is at amino acids 36 to 81 of the

sequence (counting from the NH<sub>2</sub> terminal end) (Nalefski *et al.*, *J. Biol. Chem.*, 269, 18239-18249). There are no known areas of sequence homology in the middle and C-terminal end of the cPLA<sub>2</sub> protein, i.e. there are no known areas of sequence homology between other mammalian proteins and cPLA<sub>2</sub> amino acids 82 to 749, apart from a sequence from amino  
5 acids 129 to 135 shared with pulmonary surfactant protein C precursor (Clark J. *et al.*, *J. Lipid Mediators Cell Signalling*, 12, 83-117).

The catalytic active centre of cPLA<sub>2</sub> is thought to be located in a peptide sequence which encompasses amino acid 228 (Clark J.D. *et al.*, *J. Lipid Mediators Cell Signalling*,  
10 12, 83-117).

Previous observations of phospholipase A<sub>2</sub> enzymes in the circulation have been directed at measurements in serum or plasma by substrate assay methods and have not characterised the type of the enzyme involved (Thuren T. *et al.*, *Clin. Chem.*, 31, 1985, 714-  
15 717; Gattaz W.F. *et al.*, *Biol. Psychiatry*, 22, 1987, 421-426; Gattaz W.F. *et al.*, *Biol. Psychiatry*, 28, 1990, 495-501). Serum and plasma phospholipase A<sub>2</sub> enzymes show increased activity relative to normal control subjects in the human disease schizophrenia, although again the PLA<sub>2</sub> type of the enzyme responsible has not been characterised. To date, there has been no identification of phospholipase A<sub>2</sub> protein either in or attached to  
20 red cells either physiologically or in pathology.

It has now been found that cPLA<sub>2</sub> proteins or proteins immunologically homologous to cPLA<sub>2</sub> can be detected in or on circulating red blood cells. The detection and assay of cPLA<sub>2</sub> proteins has application in the diagnosis of disease, in the monitoring  
25 of the patient response to treatment and in the development of drugs which influence the activity or concentration of cPLA<sub>2</sub>. Recognition that cPLA<sub>2</sub> proteins may be detected in or on red blood cells provides a simple and convenient method of assaying for cPLA<sub>2</sub> activity.

According to a first aspect of the present invention, there is provided an assay for  
30 detecting cPLA<sub>2</sub> or a protein immunologically homologous to cPLA<sub>2</sub>, the assay comprising use of red blood cells. The assay may also be used for quantifying the level of cPLA<sub>2</sub> in a sample of red blood cells.

As used herein, the term "a protein immunologically homologous to cPLA<sub>2</sub>" means a protein that binds specifically to an antibody that recognises an epitope or epitopes from amino acids 82 to 749 of cPLA<sub>2</sub> protein from human monocyte (U937) cells. In a preferred embodiment of the invention, the term "a protein immunologically homologous to cPLA<sub>2</sub>" means a protein that binds specifically to an antibody that recognises an epitope or epitopes from a peptide sequence which encompasses the catalytic active centre of cPLA<sub>2</sub> protein from human monocyte (U937) cells. In a further preferred embodiment, the term "a protein immunologically homologous to cPLA<sub>2</sub>" means a protein that binds specifically to an antibody that recognises an epitope or epitopes from a peptide sequence which encompasses amino acid 228 of cPLA<sub>2</sub> protein from human monocyte (U937) cells. In an alternative embodiment, the term "a protein immunologically homologous to cPLA<sub>2</sub>" means a protein that binds specifically to an antibody that has been raised against an epitope or epitopes from amino acids 82 to 749, and preferably against an epitope or epitopes from a peptide sequence which encompasses the catalytic active centre, of cPLA<sub>2</sub> protein from human monocyte (U937) cells or a synthetic peptide matching the sequence of amino acids within the range 82 to 749, and preferably a peptide sequence encompassing the catalytic active centre, of cPLA<sub>2</sub> protein from human monocyte (U937) cells. In a further alternative embodiment, the term "a protein immunologically homologous to cPLA<sub>2</sub>" means a protein that binds specifically to an antibody that has been raised against an epitope or epitopes from a peptide sequence which encompasses amino acid 228 of cPLA<sub>2</sub> protein from human monocyte (U937) cells, or a synthetic peptide matching the sequence of amino acids from a peptide sequence which encompasses amino acid 228 of cPLA<sub>2</sub> protein from human monocyte (U937) cells.

According to a second aspect of the present invention, there is provided a method of diagnosis of a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells. The method may further comprise determining the level of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells and, optimally, comparing the level with a control level.

As used herein, the term "highly unsaturated fatty acids" includes all fatty acids released by action of the cPLA<sub>2</sub> enzyme. In an embodiment of the invention the term "highly unsaturated fatty acids" includes fatty acids having 3 or more carbon-carbon double bonds. In particular, the term includes the essential fatty acids, particularly the fatty acids of the group comprising dihomogammalinolenic acid (DGLA; 8,11,14- eicosatrienoic acid),  
5 arachidonic acid (AA; 5,8,11,14-eicosatetraenoic acid), adrenic acid (7,10,13,16-  
docosatetraenoic acid), 4,7,10,13,16-docosapentaenoic acid, stearidonic acid (SA; 6,9,12,15-octadecatetraenoic acid), 8,11,14,17-eicosatetraenoic acid, eicosapentaenoic acid (EPA; 5,8,11,14,17-eicosapentaenoic acid), docosapentaenoic acid (DPA; 7,10,13,16,19-  
10 docosapentaenoic acid) and docosahexaenoic acid (DHA; 4,7,10,13,16,19-  
docosahexaenoic acid).

The invention is particularly concerned with diseases in which dysfunction of cell signalling systems involving arachidonic acid, dihomogammalinolenic acid,  
15 eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid is implicated. More particularly, the invention is concerned with diseases in which dysfunction of cell signalling systems involving arachidonic acid is implicated.

According to a third aspect of the invention, there is provided a method of  
20 monitoring the effectiveness of medication administered to a patient suffering from a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells. The method may, for example, comprise the steps of administering to a patient a compound to be tested,  
25 determining the level of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells and optimally comparing the level with a control level or a level or levels determined at an earlier stage of the medication regime or before the medication regime commenced.

30 According to a fourth aspect of the invention, there is provided a method of drug development for a disease in which dysfunction of the cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells. The



method may, for example, be used in the screening of compounds for use in the treatment of a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, the method comprising the steps of administering to a patient or test animal a compound to be tested, determining the level of cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells and optimally comparing the level with a control level.

The disease conditions in which the identification of cPLA<sub>2</sub> proteins or proteins immunologically homologous to cPLA<sub>2</sub> in or on red cells may be useful include:

- 1) schizophrenia, in which increased cPLA<sub>2</sub> activity is a proposed mechanism in disease development;
- 2) bipolar or manic depressive illness, in which cPLA<sub>2</sub> abnormality may be present;
- 3) cachexia, in which tumour necrosis factor promotes cPLA<sub>2</sub> activity;
- 4) brain injury, including stroke and mechanical injury, in which cPLA<sub>2</sub> may be released from damaged membranes or as part of the process of apoptosis; and
- 5) any other disease or disease process in which cPLA<sub>2</sub> activity or concentration is increased or decreased from normal levels, particularly a disease or disease process in which cPLA<sub>2</sub> activity or concentration is increased.

The assays and methods of the invention may comprise the steps of collecting a sample of blood from a subject, and detecting the proteins *ex vivo*. Preferably, the assays and methods further comprise one or more of the steps of separating the red cells from the other blood components, disrupting the red cells by a method such as sonication, nitrogen cavitation, freezing or lysis, and detecting the proteins either directly or following a protein separation technique.

In a preferred embodiment, the assays and methods of the invention comprise the detection in or on red blood cells of one or more proteins from one or more of three particular groups of proteins. The proteins are grouped by apparent molecular weight as measured using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) with western blot and can be designated as follows:

**Group A**, comprising one or more proteins of apparent molecular weight in the range of 90 to 105 kDa;

**Group B**, comprising more or more proteins of apparent molecular weight in the range of 70 to 80 kDa;

- 5 **Group C**, comprising one or more proteins of apparent molecular weight in the range of 50 to 60 kDa.

It is considered that the proteins in a given group may be structurally related, small variations in apparent molecular weight between the proteins in a given group possibly  
10 being due to the extent of phosphorylation. However, it is not intended that the scope of the invention be limited by this theory. Variations in apparent molecular weight among proteins of a given group may simply be due to variations in the length of the amino acid sequence of the protein or protein fragment.

15 These proteins react immunologically with antibodies to cPLA<sub>2</sub>. The proteins can be identified by an antibody or antibodies which react with an epitope present in a region of the cPLA<sub>2</sub> protein other than the calcium-binding portion of the protein. Protein detection and identification is discussed in more detail below.

20 It is considered that the proteins or protein fragments isolated from in or on red blood cells may be the same as the cPLA<sub>2</sub> protein, or fragments thereof, found in human monocyte (U937) cells. Thus, it is considered that the proteins having apparent molecular weights in the range of 90 to 105 kDa, in the range 70 to 80 kDa and in the range 50 to 60 kDa may be either intact cPLA<sub>2</sub> or major components of cPLA<sub>2</sub>. However, it is not  
25 intended that the scope of the invention be limited by this theory.

According to a further aspect of the invention there is provided a protein obtainable by isolation from red blood cells, said protein being immunologically homologous to cPLA<sub>2</sub>. Said protein may exist in the body in the form of a protein having a molecular  
30 weight in the range 90 to 105 kDa or in the form of a protein having a molecular weight in the range 70 to 80 kDa or in the form of a protein having a molecular weight in the range 50 to 60 kDa.

### Protein Separation

The proteins of the cPLA<sub>2</sub> enzyme are typically separated from the other proteins of red blood cells by SDS PAGE but a variety of other protein separation techniques may be used, including native polyacrylamide gel electrophoresis and various column separation techniques such as fast protein liquid chromatography and variants of sepharose or other commercially available columns. Two dimensional electrophoretic techniques may also be used.

### Protein Detection

The detection of cPLA<sub>2</sub> proteins or proteins immunologically homologous to cPLA<sub>2</sub> can be achieved by a wide range of protein detection procedures including:

- 1) enzyme linked immunoassay, radioimmunoassay, luminescence immunoassay, fluorescence immunoassay or any other variant of competitive or tagged antibody immunoassay;
- 2) immunofluorescence or other immunoassay on red blood cells in smears, droplets, films, fixed histological sections or tissue samples; and
- 3) *in vitro* tests in which the protein is recognised by a specific polyclonal or monoclonal antibody and the specific antibody is itself recognised in a Coombs-type test.

In a preferred embodiment, the proteins are detected using either a polyclonal or a monoclonal antibody. Table 1 provides examples of antibodies used in the assays of the present invention for the detection of cPLA<sub>2</sub> proteins or proteins immunologically homologous to cPLA<sub>2</sub>.

Suitable antibodies for use in the present invention can also include, for example, a polyclonal antibody raised against an epitope of the peptide chain comprising amino acids 730 to 749 of cPLA<sub>2</sub> from U937 cells; an antibody raised against a mid-molecule sequence encompassing the catalytic active site of cPLA<sub>2</sub> from U937 cells; and an antibody raised against an epitope from the N-terminal part of the sequence (amino acids 1 to 216), which has known sequence homology with other proteins as mentioned above or an antibody raised against another epitope or epitopes from the amino acid sequence of cPLA<sub>2</sub> protein from U937 cells between amino acids 82 and 749.

**Table 1**

ANTIGEN	TYPE	PRODUCTION METHOD	SOURCE	DETECTS cPLA <sub>2</sub> -LIKE PROTEINS IN RED CELLS
Human cPLA <sub>2</sub> Synthetic Peptide From mid molecule	Polyclonal IgG	Raised in Rabbits	Cayman Chemical	Yes
Human cPLA <sub>2</sub> Synthetic Peptide C Terminal Domain amino acids 725-749	Polyclonal IgG	Raised in Sheep	The Binding Site	Yes
Human cPLA <sub>2</sub> Synthetic Peptide to amino terminal amino acids 1-216	Monoclonal IgG	Mouse	Santa Cruz Biotechnology	Yes

- The recognition of cPLA<sub>2</sub> proteins or proteins immunologically homologous to
- 5 cPLA<sub>2</sub> in an accessible component of blood opens the way to their use as follows:
    - a) Diagnostic use by direct immunoassay of their level.
    - b) Use in a diagnostic sense indirectly as part of a Coombs-type test.
    - c) Recognition by immunofluorescence on or in red blood cells on slide or smear or tissue preparation.
    - 10 d) Use in an immune precipitation or other reaction to recognise cPLA<sub>2</sub> proteins or proteins immunologically homologous to cPLA<sub>2</sub> in whole blood samples in near-patient testing.
    - e) Their use in monitoring of treatments which are directed to suppress phospholipase A<sub>2</sub> activity or concentration.
    - 15 f) Their use in the research for agents which suppress phospholipase A<sub>2</sub> activity or concentration for drug discovery.

The invention will now be described in detail with reference to the following examples. It will be appreciated that the invention is described by way of example and modification of detail may be made without departing from the scope of the invention.

## 5 EXAMPLES

The collection of samples for protein separation and detection were performed as follows:

- 1) Samples of venous blood (4 ml) were collected by venepuncture using EDTA at standard concentration for anticoagulation (Vacutainer ®). Other appropriate anticoagulants known to persons skilled in the art may also be used.
- 2) Within 3 minutes of the sample being withdrawn, protease inhibitors were added. To 4 mls of blood were added 0.5 ml of a protease inhibitor cocktail, freshly prepared, as follows:  
To 3 mls of Aprotinin (Trasylol ®) concentration 10,000 Kallikrein inactivator units per ml was added 2 mg phenylmethanesulphonyl fluoride (PMSF) and 0.5 mg leupeptin. Other appropriate inhibitor preparations known to persons skilled in the art may also be used.
- 3) The whole blood sample was then centrifuged, at 3000 rpm (1,000 g), for 10 minutes. Plasma was removed and the layer of white cells and platelets (the buffy coat) removed by plastic pipette, to leave the red cells in the tube.
- 4) A volume of ice cold phosphate buffered saline PBS pH 7.4 or other appropriate buffer, equal in volume to the red cells was added and red cells and buffer were mixed by inversion.
- 5) The cells in buffer were then centrifuged, at 1,000 g, repeating steps 3 and 4 three times, providing washed red cells.
- 6) The washed red cells in 0.5 ml aliquots were suspended in 0.5 ml of buffer at 4°C, prepared as follows: 0.37 g KCl, 0.74 g disodium EDTA, 3.0 g Tris, 9 g NaCl in 1

litre of water, adjusted to pH 7.4 with HCl. (Kramer *et al. J. Biol. Chem.* 266, 5268-5272).

- 7) These samples were stored frozen at  $-80^{\circ}\text{C}$  or used directly after brief freezing to provide lysis for SDS polyacrylamide gel electrophoresis or for immunoassay in a calibrated assay.

Protein separation and detection was achieved using SDS PAGE as described below.

10

In SDS PAGE, the sample was treated with a volume of sample buffer, comprising Tris buffer pH 6.8 containing 6% SDS, 0.5% dithiothreitol and 20 ml glycerol per 100 ml. (Alternative buffers known to persons skilled in the art may be used). Lysate was added to give a 5-fold or 2-fold dilution of the lysate in sample buffer. The sample was transferred to the standard SDS 7.5% polyacrylamide gel for electrophoresis.

The test membrane was reacted with polyclonal antibody to mid-molecule cPLA<sub>2</sub> raised in rabbit and then anti rabbit IgG antibody raised in swine which has been coupled to horse raddish peroxidase to demonstrate the presence of the cPLA<sub>2</sub> antigen. The blank membrane was reacted with anti rabbit IgG antibody raised in swine which has been coupled to horse raddish peroxidase. Any bands observed on the blank membrane are due to the presence of antigens other than the cPLA<sub>2</sub> antigen.

Figure, 1a and 1b show two western blot analyses (figure 1a is the test membrane; figure 1b is the blank membrane) on the following samples:

- Lane 1: Molecular weight markers  
Lane 2: Cell cytosol from insect cells infected with Baculovirus expressing cytosolic PLA<sub>2</sub> Type IV.  
Lanes 3-12: Patient samples. The samples were taken from schizophrenic patients on clozapine (lanes 4, 7, 9 and 10), schizophrenic patients (lanes 8 and 12) and from control patients (lanes 3, 5, 6 and 11).

The patient samples on the test membrane show three band types grouped by molecular weight as described above:

5      **Group A:**      one band is observed above the 97 kDa molecular weight marker, having a molecular weight of about 100 to 105 kDa, and one band is observed below the 97 kDa molecular weight marker, having a molecular weight of about 90 to 95 kDa;

10      **Group B:**      a single band is observed with molecular weight around 70 kDa;

15      **Group C:**      three bands are observed with molecular weights around 60 kDa. Two of the bands in this molecular weight group are believed to be cPLA<sub>2</sub> proteins. The most distinct of these also appears on the blank membrane, has a less specific immunological response and may not be a cPLA<sub>2</sub> protein.

The samples from patients on clozapine showed suppression of the cPLA<sub>2</sub> bands.

20      In summary, the red cell lysates contained five proteins, two in the range of about 90 to about 105 kDa, one in the range of about 70 to about 80 kDa, and two in the range of about 50 to about 60 kDa, which reacted specifically in SDS PAGE western blot analysis with polyclonal antibody prepared against mid-molecule sequences of cPLA<sub>2</sub>.

25      As noted above the bands grouped in Groups A, B and C may represent, respectively, the intact cPLA<sub>2</sub> molecule in differing stages of phosphorylation and two groups of peptide sequences of cPLA<sub>2</sub> proteins which have lost amino acids by protein degradation. However, as stated above, it is not intended that the scope of the invention be limited by this theory. The bands grouped in Groups A, B and C could also represent intact cPLA<sub>2</sub> and different fragments of cPLA<sub>2</sub> differing in the length of amino acid sequence.

## CLAIMS

1. An assay for detecting cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) or a protein immunologically homologous to cPLA<sub>2</sub>, the assay comprising use of red blood cells.  
5
2. An assay according to claim 1 for use in the diagnosis of a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated.  
10
3. An assay according to claim 1 for use in monitoring the effectiveness of medication administered to a patient suffering from a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated
- 15 4. An assay according to claim 1 for use in drug development for a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated
5. A method of diagnosis of a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells.  
20
6. A method of monitoring the effectiveness of medication administered to a patient suffering from a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells.  
25
- 30 7. A method of drug development for a disease in which dysfunction of cell signalling systems involving highly unsaturated fatty acids is implicated, said method comprising the detection of cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) protein or a protein immunologically homologous to cPLA<sub>2</sub> in or on red blood cells.



8. An assay or method according to anyone of claims 1 to 7 wherein the red blood cells are isolated from the human body.
- 5 9. An assay or method according to anyone of claims 2 to 8 wherein said disease is a disease or disease process in which cPLA<sub>2</sub> activity or concentration is altered from normal levels.
- 10 10. An assay or method according to anyone of claims 2 to 8 wherein said disease is a disease or disease process in which cPLA<sub>2</sub> activity or concentration is increased.
11. An assay or method according to any one of claims 2 to 10 wherein the disease is schizophrenia, bipolar or manic depressive illness, cachexia or brain injury.
- 15 12. An assay or method according to claim 11 wherein the brain injury is stroke or mechanical brain injury.
13. An assay or method according to any one of claims 1 to 12 wherein the cPLA<sub>2</sub> protein or the protein immunologically homologous to cPLA<sub>2</sub> has a molecular weight in the range 90 to 105 kDa or in the range 70 to 80 kDa or in the range 50 to 60 kDa.
- 20 14. An assay or method according to any preceding claim comprising the steps of collecting a sample of blood from a subject and detecting the proteins *ex vivo*.
- 25 15. An assay or method according to claim 14 further comprising one or more of the steps of separating the red cells from the other blood components, disrupting the red cells, detecting the proteins either directly or following a protein separation technique.
- 30 16. An assay or method according to claim 15 wherein the red cells are disrupted by sonication, freezing, nitrogen cavitation or lysis.

17. An assay or method according to any preceding claim wherein said proteins are detected by immunoassay.
- 5 18. An assay or method according to any preceding claim wherein said proteins are detected using an antibody or antibodies that recognise an epitope or epitopes from amino acids 82 to 749 of cPLA<sub>2</sub> protein from human monocyte (U937) cells.
- 10 19. An assay or method according to any preceding claim wherein said proteins are detected using an antibody or antibodies raised against an epitope or epitopes from amino acids 82 to 749 of cPLA<sub>2</sub> protein from human monocyte (U937 cells) or raised against an epitope or epitopes of a synthetic peptide matching amino acids 82 to 749 of cPLA<sub>2</sub> protein from human monocyte (U937) cells.
- 15 20. An assay or method according to claim 18 or 19 wherein said epitope or epitopes are from a peptide sequence which encompasses the catalytic centre of cPLA<sub>2</sub> protein from human monocyte cells.
- 20 21. A protein obtainable by isolation from red blood cells, said protein being immunologically homologous to cPLA<sub>2</sub> and having a molecular weight in the range 90 to 105 kDa or a molecular weight in the range 70 to 80 kDa or a molecular weight in the range 50 to 60 kDa.
- 25 22. A diagnostic kit comprising means for disrupting red blood cells and further comprising an antibody or antibodies to a protein obtainable by isolation from red blood cells, said protein being cPLA<sub>2</sub> protein or a protein immunologically homologous to cPLA<sub>2</sub>.
- 30 23. A diagnostic kit according to claim 22 wherein said antibody or antibodies is/are raised against an epitope or epitopes from amino acids 82 to 749 of cPLA<sub>2</sub> protein from human monocyte (U937) cells.

24. A diagnostic kit according to claim 22 wherein said antibody or antibodies is/are raised against an epitope or epitopes from a peptide sequence which encompasses the catalytic centre of cPLA<sub>2</sub> protein from human monocyte (U937) cells.
- 5 25. A diagnostic kit according to claim 22, 23 or 24 wherein said means for disrupting red blood cells is a means for lysing red blood cells.



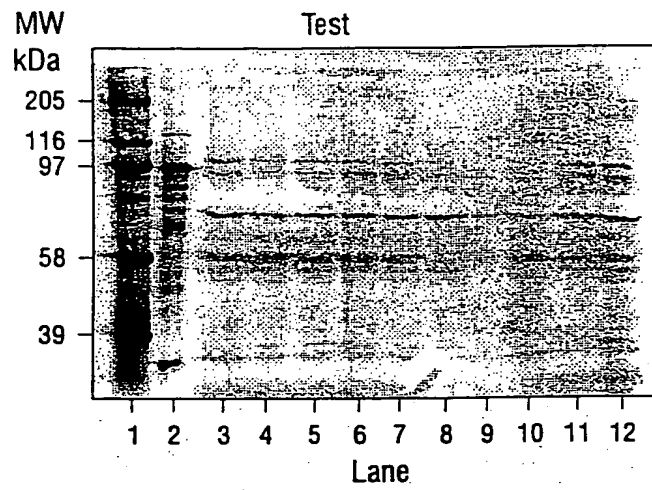


Fig 1a

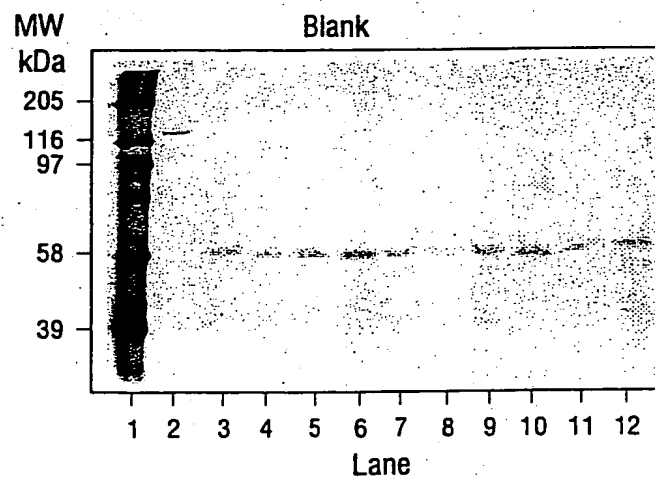


Fig 1b

Form 23177 : 7/4/00

Agent : Corporals L Ransford.